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File: USPT

Sep 15, 1998

DOCUMENT-IDENTIFIER: US 5808028 A

TITLE: Molecular clone of a P58 receptor protein and uses thereof

Brief Summary Text (2):

The p58 receptor of NK cells is a family of cell surface molecules with a molecular mass of about 58,000 which is involved in the recognition of target cells by NK cells. The p58 receptor contributes to the specificity of NK cells in their recognition of major histocompatibility complex (MHC) class I molecules on target cells. The specific recognition of MHC molecules mediated by the p58 receptor protects the target cell from lysis by the NK cell. Conversely, absence of the appropriate MHC class I molecule on target cells leads to NK-mediated lysis.

Brief Summary Text (3):

Prior to the present invention, however, no members of the p58 family have been purified sufficiently to permit their isolation and characterization. This is because it is impossible to grow large enough populations of the NK cells to permit isolation of sufficient amounts of p58 receptor protein for sequencing. Furthermore, the known NK cell lines express high levels of Fc receptor which could interfere with immunoaffinity purifications utilizing monoclonal antibodies directed to the p58 receptor protein, since these antibodies also bind to the Fc receptor.

Brief Summary Text (4):

In particular, Moretta, et al. (1990) described the use of a monoclonal antibody designated GL183 to immunoprecipitate either a 58 Kd band or a broad 55-58 Kd band from NK clones which had been labeled with ¹²⁵I (Moretta, et al. J. Exp. Med. 171: 695-714 (1990); Moretta, et al. Advances in Immunology 55: 341-380 (1994)). However, Moretta, et al. did not sufficiently purify the alleged p58 receptor protein to permit its isolation and characterization, and could not have done so utilizing the particular NK cells described therein, or known NK cells.

Brief Summary Text (5):

This is because NK cells described in Moretta, et al., as most NK cells, cannot be grown to large enough populations to permit isolation of sufficient amounts of p58 receptor protein for sequencing. Specifically, about 1.times.10.¹⁰ NK cells would have to be obtained in order to purify an amount of pure p58 protein sufficient for sequencing. However, NK cells cannot be expanded much beyond 1.times.10.⁷ cells since they do not grow well in culture.

Brief Summary Text (6):

Moreover, the NK cell lines described in Moretta, et al. express high levels of Fc receptor which could interfere with immunoaffinity purifications utilizing the GL183 monoclonal antibody, since this antibody also binds to the Fc receptor. Furthermore, the inventors of the present invention found that the p58 and Fc receptors (CD16) have very similar isoelectric points and relative masses, which could make their separation difficult.

Brief Summary Text (7):

The inventors of the present invention overcame the potential problems in the art by employing a long term NK cell line designated NK 3.3 which, unlike previously identified NK clones, grows well under long-term culture conditions, can be readily expanded to sufficient numbers, and expresses very low levels of the Fc receptor. Accordingly, the inventors of the present invention were the first to purify a

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